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CLINICAL AND HORMONAL MILIEU OF 9 PATIENTS WITH PRIMARY GROWTH HORMONE INSENSITIVITY SYNDROME AND THEIR RESPONSE TO IGF-I GENERATION TEST

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Abstract- Primary growth hormone insensitivity syndrome (GHIS) is a rare entity which can be due to defects in growth hormone (GH) receptor that is called type 1 Laron syndrome (T1LS) or post receptor defects (type 2 Laron syndrome). The aim of study was determining the clinical and hormonal milieu of the patients with primary GHIS and their response to IGF-I (insulin like growth factor-I) generation test (IGT). GH, IGF-I, IGF-II, IGF binding protein 1 and 3 (BP-1 and BP-3), GH binding protein (GHBP) and anti-GH antibody were detected by ELISA and RIA methods. IGF-I and BP-3 were measured before and after IGT**.** Nine patients (8 males, 1 female) (mean age ± SD, 6.4 ± 5 years) with severe short stature and high GH level were studied. Height SDS was - 8.5 ± 2.6. In 7 patients GHBP was zero, IGF-I and BP-3 were low and did not increase after IGT, so they had T1LS. Two brothers did not show the hormonal milieu of GH receptor defect, and were called non Laron syndrome (NLS). Birth weight in patients with T1LS and NLS was 3.65 ± 0.2 Kg and 1.65 ± 0.2 Kg, respectively (P = 0.001). All of the patients had typical clinical feature of GH-deficiency, but nasal bridge depression and microphallus were not seen in NLS. GH treatment of NLS, normalized their growth velocity, but without catch up growth. In conclusion IGT can differentiate Laron syndrome from other types of short stature. GH and IGF-I of fetus have no role in intrauterine growth.

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Key words: Growth hormone insensitivity syndrome, Type 1 Laron syndrome, IGF-I generation test, IGF-I, IGF binding protein-3, IGF binding protein-1, IGF-II

INTRODUCTION

Growth hormone insensitivity syndrome (GHIS) can be due to polymorphic molecular defects in the growth hormone (GH) receptor leading to inability to generate endogenous insulin like growth factor-I (IGF-I) that is called type 1 Laron syndrome (T1LS) or to post receptor defects named as type 2 Laron syndrome (T2LS). The first report on T1LS was in 1966 by Laron (1). These patients have typical clinical features of GH deficiency consisting of frontal bossing, nasal bridge depression and triangular face due to underdeveloped mandible, crowded teeth, high pitched voice, microphallus and chubby body (2). Clinical and laboratory study of these patients opens perspectives in the investigation of GH and IGFs action (2-4).

Clinical and laboratory investigation and IGF-I generation test were performed in a group of patients with severe short stature and high GH level. The aim of study was determining the clinical

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and hormonal milieu of the patients with primary GHIS and their response to IGF-I generation test (IGT).

MATERIALS AND METHODS

Nine patients (8 males, 1 female) were studied. The age of patients was from 4 months to 17.58 years (mean ± SD) (6.4 ± 5 yr). All of the patients were products of consanguineous marriages and the parents were first cousin. They were among 21 siblings (12 males, 9 females) belonging to 5 families. Two of these families were relatives. Routine biochemical studies were done. GH was measured at different times of day (0700 h, 1200 h, 1600 h and 2000 h) in the Institute of Endocrinology and Metabolism in Tehran with a commercial radioimmunoassay (RIA) kit (Spectria, Orion Diagnostica, Espoo, Finland). Intra assay coefficient of variation (CV) for 2.5 ng/mL and 40.58 ng/mL was 6.1% and 4.4%, respectively, and inter assay CV for 1.36 ng/mL and 12.5 ng/mL was 13.7% and 4% respectively.

Skeletal survey was done for case 2 and 3. Bone age was determined by Tanner-Whitehouse method according to Atlas of Greulich and Pyle, and skull X-ray was done for all of the patients. Weight height index (WHI, [patient weight/weight for height age] x 100) was determined and GVI ([patients growth velocity/normal growth velocity for age] x 100) was calculated before and after treatment with GH for patients 2 and 3. Skin fold thickness was measured in different parts of body as indicator of subcutaneous fat tissue. IGF-I, IGF-II, BP-1, BP-3, GHBP and anti GH antibody were measured as baseline. IGT was performed according to instruction of Kabi Pharmacia in a collaborative study as "Extended screening for definition of GHRD and GH-gene deletion". IGF-I and BP-3 were measured at day 5 and 8 after low dose and high dose subcutaneous GH administration.

Method of IGT

Seven rhGH injections were given to the patients in the evening, during 7 consecutive days (except day 1 which for practical reason, were given in the morning). The dosages were as follow: Day 1–4, 0.1 IU/kg/day subcutaneously (SC); day 5–7, 0.2 IU/kg/day SC. Blood samples for measurement of IGF-I and BP-3 were drawn in the morning of day 1 (pre-test), day 5 (within 12 h after the latest injection) and day 8 (within 12 h after the latest injection). The subjects were fasted for 10 hours before the samplings. In the morning of day 1, samples for measurement of GH, IGF-II, IGFBP-1, and GHBP and anti GH antibody were also provided. Blood samples were drawn into standard tubes without anticoagulants and left at room temperature > 30 minutes to coagulate except for the samples of GHBP that were drawn in the tubes with anticoagulants. After centrifugation the serum and plasma samples were stored in a deep freezer -70 °C in separate plastic tubes with specific labels until shipped on dry ice to Sweden by fast mail. All of these laboratory analyses were performed at Kabi Pharmacia or affiliated laboratories in Stockholm. These GH measurements were performed by immunosorbent assay (ELISA) (WHO 66/217), the measuring range of the assay was 0.4–12.5 mU/L. At concentration of 1.1 mU/L, the intraassay and interassay coefficients of variation (CV) were 3.0% and 8.0%, respectively. At 6.2 mU/L, the intraassay and interassay CV were 6.5% and 9.0%, respectively. The assay had low cross reactivity against hTSH (0.28%), hFSH (0.03%), hLH (< 0.001%), hPRL (0.007%), hCG (< 0.002%) and hPL (0.47%). Method of measurement of IGF-I was competitive RIA. At a concentration of 202 ng IGF-I/mL the intra-assay and inter-assay coefficients of variation were 3.1% and 10.0%, respectively. The IGF-I standard, DSQ 93, was an in-house standard calibrated against a WHO standard. The assay had a cross reactivity for IGF-II of about 1%. For insulin, proinsulin and smaller synthesized IGF-I fragments (1-10, 19-30, 30-40 and 57-70, respectively) the cross-reactivity was negligible.

RESULTS

The anthropometric characteristics of patients are illustrated in table 1. Biochemical profile and thyroid function tests were normal (Table 1).

According to the results of laboratory tests, 7 patients had T1LS and 2 brothers did not have LS that is called non-LS (NLS) who are patients 2 and 3. Mean of birth weight in T1LS and NLS was 3.65 ± 0.2 Kg and 1.65 ± 0.2 Kg, respectively (P= 0.001); and their birth length was 48 ± 1.7 cm and 40.0 ± 0.0

Table 1. Anthropometric characteristics and biochemical profile of the patients

	Patient										
Character	1	2	3	4	5	6	7	8	9	**Mean**	**SD**
Birth weight g	3200	1800	1500	3800	3750	ND	3400	ND	ND	-	-
Birth height cm	46	40	40	ND	49	ND	49	ND	ND	-	-
Chronological age	3.047	4.019	9.022	6.691	0.365	7.447	2.639	17.759	7.619	6.512	5.074
Height at entry cm	72	71	89	79.5	54	76	67	105	81	77	14
HSDS	-7.4	-9.5	-9.67	-9.6	- 4.6	-7.6	-8.6	-10.7	-10.4	-8.45	2.6
Weight kg	8.3	7.4	11.5	10	5.5	8.3	7.0	15.5	10	9.28	2.95
WHI	90.2	83.1	88.5	92.6	122.2	83.0	104.5	91.2	89.3	93.84	12.35
Bone age yr	1.5	2.5	7	2.5	0.25	2.5	0.83	9.5	2	3.18	3
BA - HA	0.67	1.75	4.84	1.125	0.167	1.417	0.29	5.17	0.5	1.77	1.8
Sitting height cm	40	43	54	44	34	44	40	55.5	45	44.4	6.7
Arm Span cm	70	65	90.5	72.5	53	70	62.5	96	74.5	72.67	13.4
Upper/Lower seg	1.25	1.53	1.54	1.24	1.70	1.37	1.48	1.12	1.25	1.38	0.19
Arm span - height	0.69	0.66	0.70	0.72	2.40	0.60	0.75	0.82	0.62	0.89	0.57
Biochemical profile										**Normal range**	
FBS mg/dL	82	76	65	76	60	70	96	70	60	60-100	
BUN mg/dL	17	12	11	15	13	10	12	13	17	5-18	
Cre mg/dL	0.6	0.6	0.4	0.5	0.3	0.5	0.6	1	0.8	0.3-0.7	
Na mEq/L	138	143	137	135	133	142	145	138	136	135-148	
K mEq/L	5.1	4.5	4.6	4.2	5	4.6	4.2	3.8	3.8	3.5-5.3	
Ca mg/dL	9.9	9.6	8.3	9.7	8.7	10	8.8	10.3	9.3	8.5-10.5	
P mg/dL	5.6	5.7	4.8	5.3	4.3	5.6	5.3	4	5.8	4.5-6.5	
Hb g/dL	12.7	11.3	11.7	10.8	10.3	12.1	11.5	10.9	11	10.5-14	
T_4 µg/dL	8	11.5	9.7	6	8	8.9	7.4	7.8	6.7	4.5-13	
T_3U %	26.9	28.5	26.9	25	27	32.4	29.8	28	29.4	25-35	
TSH mU/L	5.1	3.2	3.3	3.3	3.0	2.0	1.8	1.5	0.5	0.3-5.6	
FTI	2.1	3.1	2.5	3.4	2.8	2.8	2.3	3.0	1.9	1.3-4.4	
Alk P U/L	170	220	300	417	130	378	515	300	480	130-600	

Abbreviations: CA, chronological age; H, height; W, weight; WHI, weight height index; BA-HA, bone age minus height age; SH, sitting height; AS-H, arm span minus height; U/L, upper/lower segment.

cm respectively. Midparental height in T1LS and NLS was 167 ± 7 cm and 161 cm, respectively. WHI was < 100 in all of the patients except 2 patients in T1LS group. Bone age was more than height age in all of the patients.

All of the patients had typical clinical features of GH-deficiency including frontal bossing, small mandible, and disproportionate face to skull and high pitched voice. Seven patients with T1LS had nasal bridge depression, but it was not seen in NLS, especially in the elder brother. Six boys with T1LS had microphallus, but the genitalia was normal in patients with NLS. Subcutaneous fat tissue was well developed mainly in truncal and limbs areas in T1LS, but was more in truncal area in NLS (Table 2).

Table 2. Skin fold thickness of the study patients

Patient	Age	BC	TC (MNR)	SS (MNR)	SI
1	3.047	8	13 (10)	9 (6)	8
2	4.019	4	8 (9.5)	6 (5.5)	7
3	9.022	7	9 (8.5)	8 (5.5)	7
4	6.691	11	15 (8.2)	8 (5.2)	10
5	0.365	9	11.5 (10)	9 (8)	12
6	7.447	7	12 (9.8)	7 (6)	6.5
7	2.639	9	13 (10.2)	13 (6)	12
8	17.759	12	16 (8.6)	15 (9.5)	12
9	7.619	11.5	13 (8.2)	10 (5.2)	10
Mean	6.512	8.72	12.28	9.44	10.28
SD	5.074	2.56	2.56	2.88	2.5

Abbreviation: BC, biceps; TC, triceps; MNR, mean of normal range; SS, subscapular; SI, suprailiac.

Table 3. Serum GH levels (ng/mL) in different times a day and with L-Dopa test in the patients

Patients	basal	P.L-dopa	7 AM	12 AM	4 PM	8 PM
1	17.9	54.5	19.9	10.9	9.2	18.6
2	12	20	12	2	1	2
3	15	50	15	4.6	1.3	1.6
4	39	55	110.5	41	14	10.5
5	ND	ND	240.6	139.3	306.3	116.3
6	35	83	29.2	39.2	17.1	27.1
7	31	50	29.4	17.6	20	17.5
8	ND	ND	117.9	127.9	86.5	64.2
9	11	50	46.8	75.5	55.2	111.4

Abbreviation: P.L-Dopa, post L-Dopa provocative test.

GH as basal and after L-Dopa provocative test was high in all of the patients. GH was high at different times a day except in patient 1 at 1600 h and in NLS at 1200 h, 1600 h, and 2000 h (Table 3).

IGF-I and BP-3 were low before and after IGT, and GHBP was zero in T1LS. In NLS, IGF-I, and BP-3 rose after IGT and GHBP was detectable (Table 4).

The 2 brothers with NLS were treated with 0.7 U/kg/week divided to 7 subcutaneous injections before bedtime for 1 year. Growth velocity index of patients 2 and 3 was 55% and 57%, respectively, before treatment, and it increased to 91% and 100% respectively after treatment, but they did not have catch up growth. Skeletal survey in patients with NLS was normal. Skull X-ray of all the patients was normal.

DISCUSSION

The primary GH–binding protein in humans is derived by proteolytic cleavage from the extracellular domain of the GH receptor. Therefore, it is not surprising that GHBP was found to be absent from the circulation of patients with GH receptor deficiency (5-7). GHBP was zero in 7 of our patients, so their diagnosis was T1LS. It was present in patients 2 and 3 (NLS). Basal IGF-I and BP-3 were low in T1LS and did not rise by IGT, but they were normal in NLS and increased by IGT. So NLS had functioning GH receptor that could produce IGF-I and BP-3.

NLS patients at the age of 4 and 9 yr had severe short stature that is evidenced from height SDS of –9.5 and -9.67, respectively. They had normal skeletal

Table 4. The results of laboratory investigations and IGT in the study patients*

Patient	GH	IGF-I (ng/mL)			IGF-II	BP-I	BP-3 (ng/mL)			GHBP	Anti
	mU/L	Pre	day 5	day 8	ng/mL	ng/mL	Pre	day 5	day 8	%	GH
1	30.4	<20	<20	<20	47	97	203	199	210	0	0
2	20	51	173	247	331	35	2206	2887	4149	19.6	0
3	46.3	227	377	371	463	19	4744	4857	4949	36.1	0
4	32.8	<20	<20	<20	39	119	262	246	209	0	0
5	124.7	<20	<20	<20	153	362	462	253	544	0	0
6	113.6	<20	<20	<20	67	78	326	386	407	0	0
7	56.1	<20	<20	<20	55	190	248	207	261	0	0
8	24.3	<20	<20	<20	147	68	438	464	513	0	0
9	17.9	<20	<20	<20	57	215	277	231	202	0	0

Abbreviations: GH, growth hormone; IGF, insulin like growth factor; BP-I and BP-3, IGF binding protein I and 3; GHBP, GH binding protein; Anti GH, anti GH antibody.
*Normal values are: GH, up to 10 mU/L; IGF-I, males 197 ± 15 ng/ml and females 182 ± 14 ng/ml; BP-I, 9–47 ng/ml; BP-3, 1200–3000 ng/ml.

survey and did not have any dysmorphic appearance or systemic illness. Their growth velocity normalized with growth hormone, but did not catch up growth.

Conditions of insensitivity to IGF-I action exists and includes abnormalities in IGF transport and clearance that would alter presentation of IGF to its receptor (8, 9) or primary defects of IGF receptor production or responsiveness (10). In the African pygmies, a series of studies (11) demonstrated extreme insensitivity to the in vitro growth-enhancing effects of IGF-I. In our study 2 patients had severe short stature with no special feature of any syndrome. IGT and laboratory investigation showed, that the GH receptor was present, and was capable of producing IGF-I and IGF-BP3. Because they responded to exogenous GH although not dramatically, it does not seem that they have IGF-I insensitivity, so clearing the cause of their growth retardation needs molecular investigations. Patients with GH receptor defects that were described by Laron (1) have typical clinical feature of GH deficiency as noted in our T1LS patients, but NLS patients did not have microphallus and nasal bridge depression, so these two signs are more specific for GH deficiency or insensitivity. WHI was normal in these children but subcutaneous fat was increased so it is better indicator of fat accumulation in these children. NLS patients had normal IGF-I and low birth weight, but the patients with T1LS had normal birth weight in spite of low IGF-I, thus fetal IGF-I do not play an important role on fetal growth.

In conclusion, intrauterine growth retardation was not seen in patients with type 1 Laron syndrome. Nasal bridge depression and microphallus were specially seen in T1LS. Hormonal milieu and the response to IGT were evaluated in patients with severe short stature and high growth hormone.

Conflicts of Interests

We have no conflicts of interest.

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